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Research Article

MUCOR MYCOSIS AND OTHER FUNGAL INFECTIONS IN COVID-19 PATIENTS DURING SECOND WAVE OF PANDEMIC AT A TERTIARY CARE HOSPITAL

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ABSTRACT

Objectives: In India, there has been a sudden Surge in Mucor mycosis cases during second wave of COVID-19, made the Government of India declare it as a notifiable disease. In this background, we did a study to know the fungal etiology of the suspected Mucor mycosis cases and to know the positivity rate of Mucor mycosis in COVID-19 patients during the second wave of the COVID-19 pandemic.

Methods: In a retrospective study, we examined different samples from 112 Covid-19 positive, in-patients with suspected Mucor mycosis. A history of risk factors was taken. Potassium hydroxide (KOH) mount was done for all samples followed by culture on Sabouraud's dextrose agar.

Results: 81.25% of total cases were from 31 to 60 years of age group. Diabetes mellitus, steroid therapy, and Intensive care unit admissions were the risk factors observed. Fungal filaments were observed in 37 samples in KOH mount (33%) and culture was positive in 45 samples (40.17%). Mixed infection was seen in two cases (4.44%). The most common fungus isolated was Mucor in 23 samples (48.93%) followed by *Aspergillus* in 7 cases (14.89%; *Aspergillus niger* in four cases, *Aspergillus fumigatus* in 2 cases and *Aspergillus flavus* in 1 case) and Candida spp in 6 cases (12.76%). Among mixed infections, Mucor and *A. niger* in one case and Candida and *A. niger* in another case were isolated. Rhyzopus, Syncephalastrum, *Penicillium,* and *Fusarium* were the other fungi isolated.

Conclusion: Mucorales were the most common species isolated (63.82%) followed by *Aspergillus* species. High suspicion and diagnosis of Mucor mycosis in Covid-19 patients is very important to initiate antifungal treatment, to reduce the mortality.

Keywords: COVID-19 Pandemic, Opportunistic infections, COVID-19-associated Mucor mycosis, Diabetes mellitus, Steroid therapy.

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INTRODUCTION

The disease pattern of COVID-19 can range from mild to life-threatening pneumonia with associated bacterial and fungal co-infections. Due to the associated co-morbidities like Diabetes mellitus (DM), chronic obstructive pulmonary disease, immunocompromised conditions [1], corticosteroid therapy, ventilation, intensive care unit (ICU) stay [2,3], these patients are prone to develop severe opportunistic infections, such as oropharyngeal candidiasis, pneumocystis jiroveci pneumonia, pulmonary aspergillosis, bloodstream candida infections, and rhino-orbital Mucor mycosis [4]. Mucor mycosis, a newly emerging malignancy associated with the coronavirus (COVID-19) infection, had infected at least 7,250 people in India by the 3rd week of May 2021 [5].

Fungi mostly cause Mucor mycosis are *Rhizopus* spp., *Mucor* spp., *Rhizomucor* spp., *Syncephalastrum* spp., *Cunninghamella bertholletia*, Lichtheimia (formerly Absidia) [6-8] *Cokeromyces*, Actinomucor [1], and *Apophysomyces elegans* [9].

Mucor mycosis, commonly known as "Black Fungus" which was then a rare fungal infection, has suddenly come to light post the COVID-19 pandemic, more so during the second wave in India. Individuals who lack phagocytes or have impaired phagocytic function like severely neutropenic patients are at higher risk of Mucor mycosis [10]. Mucor mycosis infections are characterized by extensive angioinvasion that results in vessel thrombosis and subsequent tissue necrosis [1] which leads to increased mortality. It thus becomes important not only for the medical fraternity but also for the general population [11] to build awareness about the same during second wave when Mucor mycosis cases in COVID-19 patients created an alarm in the country. Mucor mycosis has been declared an epidemic in several Indian states and has been classified as a notifiable disease [12].

METHODS

Samples that were received from suspected Mucor mycosis cases of COVID-19-positive patients, from Covid ward, Government General Hospital, Srikakulam were processed under strict aseptic conditions at our laboratory. A history of co-morbidities was taken. Samples (maxillary wash, Nasal wash, nasal scrapings, nasal crusts, sputum) were placed on a clean glass slide, and about 5–10 uL potassium hydroxide (KOH) solution is poured. The KOH solution completely dissolves non-fungal components while fungal hyphae and yeast cells are exposed to visualization under a microscope [13]. All samples were inoculated on Sabouraud's dextrose agar (SDA) with antibiotic gentamicin alone and incubated at 25°C in BOD for 5 days. SDA bottles were examined for growth once daily for 5 days and those showing growth were identified by, the color and appearance of colonies, lactophenol cotton blue teasing mount, and slide culture [14]. Samples were recollected from patients whose samples were negative in KOH mount, but positive for fungal culture, to exclude contamination during the process.

RESULTS

A total of 116 samples were received and four samples were rejected due to leaked samples and improper labeling. Samples were collected from 7 to 84 years age group. 31–60 years of group constituted 81.25% of total cases. 83 samples were collected from males (74.10%) and 29 were from females (25.89%) as shown in Table 1.

Samples received were nasal wash (42), maxillary wash (37), nasal swab (9), sputum (9), crusts from nasal cavity (8), and biopsy and scrapings from nasal cavity (7). DM was the most common risk factor (51.78%) followed by steroid therapy. Direct smear, i.e., KOH mount for fungal elements was positive for 37 samples (33%). Among these 37 direct smear-positive

Table 1: Age, gender, and comorbidity-wise distribution of cases

Age group	Males	Females	Total	DM		Steroid therapy		ICU admission	
				M	F	M	F	M	F
0–10	0	2	2	0	2	0	0	0	2
11–20	0	0	0	0	0	0	0	0	0
21–30	3	1	4	2	1	2	0	0	0
31–40	20	4	24	3	1	8	2	6	1
41–50	29	9	38	17	8	14	3	15	2
51–60	21	8	29	15	6	11	4	10	5
61–70	8	4	12	5	3	5	2	5	3
71–80	2	0	2	0	0	2	0	2	0
81–90	0	1	1	0	0	0	1	0	1
Grand Total	83 (74.10%)	29 (25.19%)	112	37	21	42	12	38	14
				58 (51.78%)		54 (48.21%)		52 (46.42%)	

Table 2: KOH and culture results in combination

KOH-positive and culture positive	KOH positive and culture negative	KOH negative and culture positive
32 (28.57%)	5 (4.46%)	13 (11.60%)

samples, only 32 samples yielded growth on fungal culture and in 5 KOH positive samples, we could not isolate any fungi and in 13 KOH negative samples, fungi were isolated as shown in Table 2. Out of 112 samples, culture was positive in 45 samples (40.17%). 33 of fungal culture-positive samples were from males (29.46% of total samples and 73.33% of culture-positive samples) and 12 were from females (accounts for 10.71% and 26.66% of total and culture-positive samples, respectively).

Type of fungal isolates, their number and percentage were given in Table 3. From the 45 samples that were culture positive for fungi, yielded 47 isolates, as mixed infection was seen in two cases (4.44%). The most common fungus isolated was Mucor in 23 samples (48.93%) followed by *Aspergillus* in 7 cases (*A. niger* in four cases, *Aspergillus fumigatus* in 2 cases, and *Aspergillus flavus* in 1 case) and *Candida* spp in 6 cases (speciation was not done). Among mixed infections, Mucor and *Aspergillus niger* in one case and Candida and *A. niger* in another case were isolated. Other fungi isolated were, *Syncephalastrum*, *Rhyzopus*, *Penicillium,* and *Fusarium* as shown in Table 3.

The type of sample and their positive rate regarding fungal isolates and mucor isolates was represented in Table 4.

Table 3: Name of the fungus and its percentage

Name of the Isolate	Number of Isolates	Percentage
Mucor	23	48.93
Aspergillus spp.	7	14.89
Candida	6	12.76
Rhyzopus	4	8.51
Syncephalastrum	3	6.38
Penicillium	2	4.25
Fusarium	2	4.25
Total	47	100

DISCUSSION

The 2019 novel coronavirus or severe acute respiratory syndrome coronavirus 2 started in China and soon spread over the world, becoming a pandemic. On December 1, 2020, the first report of the U.S. Centers for Disease Control and Prevention on COVID-19-associated fungal infections was released, highlighting COVID-19-associated pulmonary aspergillosis and the sporadic increase in *Candida auris* and invasive candidiasis during the ongoing pandemic. In the following months, this list was expanded to include other infections such as the COVID-19-associated Mucor mycosis (CAM) [7].

While India faces tough times during the second wave of COVID-19, adding more burden to such a challenging situation, Mucor mycosis, an invasive fungal infection, has seen a sudden surge in patients with COVID-19. One systematic review observed that CAM constitutes 0.3% of COVID-19 coinfections. As a result, Govt. of India has declared it as a notifiable disease, while several state governments have declared it as an epidemic [15]. Steroids, monoclonal antibodies, and broad-spectrum antibiotics used to treat COVID-19 disease have been shown to increase the risk of a new fungal infection or intensify an existing one. Furthermore, COVID-19 patients receiving oxygen therapy in an ICU with a humidifier are susceptible to fungal infection due to moisture exposure [9].

Some attributed the higher incidence of Mucor mycosis in India than in other countries due to macro-environmental factors such as: masks, improperly discarded Ryle's tube, Foley catheter and nebulization instruments, improperly sterilized or autoclaved reusable instruments, the central air conditioning systems in many hospitals that are breeding grounds for fungi, and all these combined with lack of cross-ventilation and humid weather, that are ideal for fungal infections [16]. The recommended treatment strategies primarily included surgical debridement and antifungal therapy using Amphotericin B and selected azoles [15].

Devang *et al*. studied 70 cases of Covid-19 patients with Mucor mycosis admitted in their hospital and among them 47 were males (67%), and 23 were females (33%) [17] where as in our study males were 74.10% and females were 25.89%. Another study reported 101 cases of Mucor mycosis where males predominate females (78.9%), almost similar to our study 74.10%. Mucor mycosis involving nose and sinuses (88.9%) was most common followed by rhino-orbital (56.7%) [18]. Sangeetha Kandasamy *et al*. reported 58 cases of Mucor mycosis and found mixed fungal infections (*Aspergillus* sp. and *Candida* sp.) in eight (14%) cases, which was higher than the present study (4.44%) [19] In the study of Anuja *et al*., KOH mount was positive for fungal elements in 62 patients and fungi isolated in 37 patients, out of total 90 patients of invasive fungal sinusitis. *Aspergillus* group and Mucorales contribute equally being found in 17 samples each [20], whereas Mucorales exceeded *Aspergillus* group in the present study.

In one study, they reported only one case of Mucor mycosis in an incidentally diagnosed Covid 19 positive case and isolated *Rhizopus* from sample obtained after sinus debridement in FESS [21], whereas 4 strains of *Rhizopus* were isolated out of 45 positive cases in this study. Another study also reported only one case report of rhino cerebral Mucor mycosis concurrent with COVID-19 pneumonia in a 41-year-old man with a history of type 1 DM [22].

DM was the common risk factor in most of the studies [4,23] in addition to IV dexamethasone [9,24,25]. as in the present study, whereas Stroke is the major complication of Mucor mycosis in one multicentric study [26]. Sinuses were the most common site of Mucor mycosis among COVID-19 patients at 79.4% with maxillary sinus (47.4%) being most

Table 4: Type of sample and percentage of positive samples and number of Mucor isolates

Type of sample	Number of samples	Number of Positive samples and percentage	Percentage of positivity	Number of Mucor isolates	Percentage among positive samples
Nasal Wash	42	11 (24.44)	26.19	8	72.72
Maxillary Wash	37	14 (31.11)	37.83	8	57.14
Nasal Swab	9	4 (8.88)	44.44	2	50
Sputum	9	5 (11.11)	55.55	2	40
Nasal Crusts	8	7 (15.55)	87.5	2	28.57
Biopsy and Scrapings from nasal cavity	7	4 (8.88)	57.14	1	25
Total	112	45		23	

commonly infected in one review [23]. 31.11% of isolates in our study were from maxillary wash, at a lesser rate than the above observation. Mucor mycosis affecting the nasal sinuses was the commonest (44%) in one multicentric study [25]. we isolated 26 fungal strains (13 were Mucor among them) from 66 nasal samples such as nasal wash, nasal swab, nasal crusts, biopsy and scrapings from nasal cavity, constitutes 57.77% out of total 47 fungal isolates.

CAM was observed in 58.1% of the pandemic cases in one study [27]. Whereas only 1.8% subjects were diagnosed with Mucor mycosis in COVID-19 patients in another multi centric study [28] In our study, it was 26.78% (30 *Mucorales* spp. were isolated from 112 samples). We diagnosed Mucor mycosis by KOH mount and culture on SDA unlike some study who used histopathological examination for diagnosis [29].

Diagnosis of CAM is challenging as the clinical and radiological features of pulmonary and disseminated Mucor mycosis are non-specific and could overlap with findings thought to be associated with COVID-19, which results in missed or late diagnosis. CAM can also be mistaken for other Angio invasive fungal infections, particularly with COVID-19-associated pulmonary aspergillosis being the predominant mould disease in COVID-19-associated acute respiratory distress syndrome [3]. In clinically suspected patients, presence of fungal hyphae, characteristic of Mucorales fungi, by direct examination in 10% KOH from sinus wash, scrapings, biopsy etc. was used for diagnosis. Mucor mycosis was subsequently proven based on microbiological culture or specific histological features from biopsy specimen [28]. Owing to the high mortality, high index of suspicion is required to ensure timely diagnosis and appropriate treatment in high-risk populations.

CONCLUSION

DM, steroid therapy and ICU admission were the common risk factors for the development of Mucor mycosis in covid-19 patients. Mucorales were the most common species isolated (63.82%) followed by *Aspergillus* species. High suspicion and early diagnosis are the crucial steps in reducing the mortality by Mucor mycosis in covid-19 patients.

AVAILABILITY OF DATA AND MATERIAL

Available with all authors.

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AUTHORS' CONTRIBUTIONS

1st Author: The conception and design of the study, analysis and interpretation of data and drafting the article. 2nd Author: The conception and design of the study and acquisition of data. 3rd Author: The conception and design of the study and acquisition of data.

CONFLICT OF INTEREST

Not applicable (No conflict of Interest for any author).

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